

Bassil DAHIYAT *et al.*
Application No.: 09/927,790
Page 2

PATENT

RECEIVED
CENTRAL FAX CENTER

DEC 22 2006

AMENDMENTS TO THE CLAIMS

Claims 1-51 (Canceled)

52. (New) A method for generating at least one non-naturally occurring variant protein with at least one desired characteristic relative to a target protein comprising:

- a) inputting the coordinates of said target protein into a computer;
- b) identifying a list of variable residue positions in said target protein;
- c) applying at least one scoring function to said variable positions to generate a primary library comprising optimized variant protein sequences;
- d) identifying a set of amino acids at each of said variable residue positions in said variant protein sequences of said primary library by using a probability distribution table;
- e) recombining non-variable and variable residue positions to generate a secondary library of protein sequences, wherein the set of amino acids at each of said variable residue positions is used to generate the library of protein sequences, and wherein at least one member of said secondary library is not found in said primary library;
- f) screening said secondary library to identify at least one non-naturally occurring variant protein with at least one desired characteristic; and
- g) synthesizing at least one non-naturally occurring variant protein with at least one desired characteristic identified in said secondary library.

53. (New) A method according to claim 52 wherein said recombining comprises:

- i) generating a set of oligonucleotide probes each encoding at least one of said variant amino acid residues;
- ii) using said probes in a polymerase chain reaction (PCR) to generate a plurality of oligonucleotide sequences, each encoding at least one of said second set of variant sequences; and,

Bassil DAHIYAT *et al.*
Application No.: 09/927,790
Page 3

PATENT

iii) producing said secondary library in host cells transformed with said oligonucleotide sequences.

54. (New) A method according to claim 52, wherein said scoring function is a van der Waals potential scoring function.

55. (New) A method according to claim 52, wherein said scoring function is a hydrogen bond potential scoring function.

56. (New) A method according to claim 52, wherein said scoring function is an atomic solvation scoring function.

57. (New) A method according to claim 52, wherein said scoring function is an electrostatic scoring function.

58. (New) A method according to claim 52, wherein said scoring function is a secondary structure propensity scoring function.

59. (New) A method according to claim 52 wherein said step c) comprises a plurality of scoring functions.

60. (New) A method according to claim 59 wherein said plurality of scoring functions includes a van der Waals potential scoring function.

Bassil DAHIYAT *et al.*
Application No.: 09/927,790
Page 4

PATENT

61. (New) A method according to claim 59 wherein said plurality of scoring functions includes a hydrogen bond potential scoring function.

62. (New) A method according to claim 59 wherein said plurality of scoring functions includes an atomic solvation scoring function.

63. (New) A method according to claim 59 wherein said plurality of scoring functions includes an electrostatic scoring function.

64. (New) A method according to claim 59 wherein said plurality of scoring functions includes a secondary structure propensity scoring function.

65. (New) A method according to claim 52 wherein said step c) utilizes Protein Design Automation to computationally generate said optimized primary variant sequences.

66. (New) A method according to any of claims 52 - 65 wherein said recombining is done computationally.

67. (New) A method according to any of claims 52 - 65 wherein said recombining is done by using gene shuffling.

68. (New) A method according to any of claims 52 – 65 wherein said recombining is done by using multiple PCR with pooled oligonucleotides.

Bassil DAHIYAT *et al.*
Application No.: 09/927,790
Page 5

PATENT

69. (New) A method according to claim 52 wherein said screening is done using a plurality of synthesized genes encoding a plurality of said variant proteins.